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AN INITIAL EXAMINATION OF MITOCHONDRIAL DNA STRUCTURE IN BURROWING OWL POPULATIONS

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ABSTRACT.—Sequence variation was examined in the cytochrome b region of the mitochondrial genome of Burrowing Owls (*Athene cunicularia*) from North and South America, and compared with the Elf Owl (*Micrathene whitneyi*), Barred Owl (*Strix varia*) and Eastern Screech Owl (*Otus asio*). Attempts to clone and sequence the control region of the mitochondrial genome resulted in sequences that appeared to be nuclear copies of that region. Cytochrome b sequences revealed a genetic split between Burrowing Owl populations from North and South America. This split may date back 2 million yr to the connection of these continents via the isthmian land bridge. Additional population structure appears to be of Pleistocene origin or more recent. Data indicate a possible North American origin for Burrowing Owls and subsequent dispersal via the land bridge to the South American continent. The depth of the split between Burrowing Owls from North and South America is consistent with species-level distinction. Additional data from nuclear markers, morphology and/or ecological indicators, such as behavior or vocalizations, will be necessary to confirm these results.

KEY WORDS: *Burrowing Owl*; Athene cunicularia; *mitochondrial DNA*; *cytochrome b*; *genetics*; *North America*; *South America*.

Analisis preliminar de la estructura del adn mitocondrial en poblaciones de Búho Cavador

RESUMEN.—La variación de la secuencia fue examinada en la región del citocromo b del genoma mitocondrial de Búhos Cavadores (*Athene cunicularia*) de Norte y Sur América, y fue comparada con las del Búho Elfo (*Micrathene whitneyi*), el Búho Barreteado (*Strix varia*) y el Búho Chirriador oriental (*Otus asio*). Los intentos para clonar y secuenciar la región de control del genoma mitocondrial dieron como resultado secuencias que parecían ser copias nucleares de esa región. Las secuencias del citocromo b revelaron una división genética entre las poblaciones de Búhos Cavadores de Norte y Sur América. Esta escisión puede datar de 2 millones de años atrás cuando se conectaron estos dos continentes por medio del puente terrestre del istmo. Otra estructura de la población parece tener origen en el Pleistoceno o mas recientemente. Los datos indican un posible origen Norteamericano para el Búho Cavador y una subsecuente dispersión hacia el continente Suramericano a través del istmo. La profundidad de la división entre los Búhos Cavadores de Norte y Sur América es consistente con el nivel de distinción a especie. Datos adicionales a partir de marcadores nucleares, morfología y/o indicadores ecológicos, al igual que comportamiento o vocalizaciones, serán necesarios para confirmar estos resultados.

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The Burrowing Owl (*Athene cunicularia*) is widely distributed throughout the New World, from southern Canada through Argentina, including the West Indies. Suitable habitat includes arid areas, savannas, and grasslands (Pregill and Olson 1981, Johnsgard 1988). Burrowing Owls nest in burrows and, depending on geographic locality, nest in burrow systems of colonial sciurids, other

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burrowing vertebrates, natural cavities, or dig their own burrows. Currently treated as a single species, the Burrowing Owl has 18 recognized subspecies described on the basis of plumage characteristics and geographic variation in size (Peters 1940).

The Burrowing Owl fossil record, although sparse, and the biogeographic history of savanna/arid habitats throughout the New World have led to the development of hypotheses about the evolution of this species. A presumed owl ancestor *Speotyto megalopeza* first appeared in the fossil record in the Pliocene in western Meade County, Kansas, U.S.A. (Ford 1966). The first recorded Burrowing Owl fossil from South America is from the Pleistocene (Vuilleumier 1985). The most extensive fossil record of the Burrowing Owl is found in the West Indies from the Pleistocene (Pregill and Olson 1981). The savanna/arid habitats evolved separately on the North and South American continents during the Cenozoic. For most of this period, the two continents were physically separated and the centers for the evolution for arid land communities were located centrally within each continent (Webb 1977). In North America, the amount of open habitat replacing forests increased substantially throughout the Cenozoic. Today, greater than 25% of the land cover of North America is nonforest (Webb 1977). The full extent of savanna and arid habitats in South America during this same period is not fully understood and remains controversial. Evidence indicates the development of flora associated with open habitats during the early Cenozoic. The presence of open country vertebrates from fossil sites dates to the same period and includes early ungulates, chinchilds, octodontids, and large grassland birds, such as rheas (*Rhea* spp.) and the carnivorous phorusrhacids (Webb 1978). Climatic shifts of alternating humid and dry periods in South America, associated with glacial and interglacial periods, contributed to the disjunct distribution of savannas, grasslands, and other xeric habitats (Haffer 1974). This likely contributed to the disjunct populations of Burrowing Owls currently found throughout the South American continent.

Based on the current disjunct distribution of some owl populations, climatic fluctuations, and evidence of historical extinctions and range reductions of Burrowing Owls in the West Indies, we hypothesized that mitochondrial DNA (mtDNA) would reveal population structure by broad geographic locality. Specifically, we predicted in North America the resident Florida subspecies (*A. c. floridana*) would be genetically distinct from western populations (*A. c. hypugaea*) because of its geographic isolation. We also predicted a genetic break would exist between populations found in North America and South America. Within South America, we anticipated finding genetic differences between broad areas because of the apparent restricted range and isolation of many populations and their presumed resident status.

METHODS

To test our hypotheses, samples from five recognized subspecies of Burrowing Owls were analyzed. Blood samples of ≤0.5 cc were collected from the western subspecies (*A. c. hypugaea*) in Nebraska, South Dakota, Oregon, California, and New Mexico. Blood samples of the Florida subspecies (*A. c. floridana*) were collected by Brian Mealy (Univ. Florida-Miami). Tissue samples were obtained from museum tissue collections for Burrowing Owls from Baja California, Mexico (*A. c. hypugaea*, $N = 1$); Providences Tucaman and Corrientes, Argentina (*A. c. cunicularia*, $N = 2$); Providence Trujuilo, Peru (*A. c. nanodes*, $N = 1$); and Providence Loja, Ecuador (*A. c. punensis*, $N = 1$). The initial analysis for broad geographic variation was conducted on one specimen from each of the widely separated populations from Nebraska, California, New Mexico, Florida, Baja California, Mexico, Argentina (Provinces Tucaman and Corrientes), Peru, and Ecuador to evaluate the potential of the selected marker to identify population structure. Samples were also obtained from the Elf Owl (*Micrathene whitneyi*), Barred Owl (*Strix varia*), and Eastern Screech Owl (*Otus asio*) for comparative purposes.

Target DNA Sequences. Our original objective was to examine two regions of the mitochondrial genome (the control region and a section of the cytochrome b region) to address geographic variation within subspecies, as well as broad-scale variation. However, attempts to use southern blotting to develop specific control region primers to address finer-scale questions resulted in sequences that appear to be nuclear copies of this region (Desmond 1997). These sequences are therefore not discussed further in this paper. Universal cytochrome b primers designed by Kocher et al. (1989) were used to amplify the 297 bp section from the cytochrome b region. Results from these sequences are the focus of this paper.

DNA Extraction. DNA was extracted from blood and tissue by digestion in 100 mM Tris pH 7.5, 100 mM EDTA pH 8.0, 100 mM NaCl, 0.5% SDS, and Proteinase K (0.5 ug/ml) overnight at 55°C. The DNA was purified by extracting once with phenol, chloroform (24:1, chloroform:isamyl alcohol) and twice with chloroform:isamyl alcohol (24:1). Each sample was then ethanol precipitated overnight, pelleted and washed twice with 70% ethanol, dried and re-suspended in a buffer of 1M Tris and 10M EDTA.

Polymerase Chain Reaction (PCR) Amplification. Amplification was performed in 50 ul reactions containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, each dNTP at 1mM, each primer at 5 uM, and 2 units of

Table 1. Numbers and percentages (in parentheses) of the four nucleotides in the cytochrome b sequences of the Burrowing Owl, Barred Owl, and Elf Owl, respectively.

	A	C	T	G
Athene cunicularia	71 (24)	106 (36)	70 (24)	49 (17)
Micrathene whitneyi	82 (28)	96 (33)	71 (24)	43 (15)
Strix varia	76 (26)	99 (33)	71 (24)	50 (17)
Otus asio	73 (25)	102 (35)	70 (24)	51 (17)

Taq. Each cycle of the polymerase chain reaction consisted of denaturation at 94°C for 1 min, annealing at 47°C for 1 min, and extension for 1 min 30 sec at 72°C. This cycle was repeated 35 times. All cytochrome b amplifications were sequenced using an automated sequencer at the Iowa State University DNA sequencing facility. Sequence results were checked manually using the program Editview (Version 1.0, Perkin Elmer).

To test against the possibility of contamination, blood samples of Burrowing Owls from Nebraska, Florida, California, and New Mexico were taken to a separate laboratory, where no avian work was being conducted. Using all new stock solutions, DNA was purified and amplified. PCR reactions were conducted under UV light as an additional precaution against contamination. All unique sequences were deposited in Genbank under the following accession numbers:

nkit 423326, 423334, 424445, 424887, 423676, 424455, 423688, 423690, 424905, 424907, 423692.

RESULTS

Cytochrome b Sequences. A single 297 bp fragment of the cytochrome b gene was consistently amplified for Burrowing, Elf, Barred, and Eastern Screech Owls, all of which were sequenced. Using the same primers, fragments of similar size have been amplified for other bird species (Kocher et al. 1989, Edwards and Wilson 1990, Birt-Friesen et al. 1992). Compared to sequences deposited in the Genbank, owl sequences were most similar to other avian cytochrome b sequences. The nucleotide content of the cytochrome b sequences were consistent with other published avian cytochrome b sequences showing a lower guanine (G) content and above average cytocine (C) content (Table 1). Also in agreement with other avian cytochrome b sequences, a third codon deficiency of G and thymine (T) was observed (Table 2).

Maximum parsimony analysis of the cytochrome b data was conducted with PAUP (version 3.1.1; Swofford and Begle 1993) using the heuristic search algorithm (Elf, Barred, and Eastern Screech Owls were designated as outgroups). Unweighted maximum parsimony analysis separated Burrowing Owls from North and South America, and bootstrap analysis (500 replications) provided strong support for this separation with a value of 100%.

Cytochrome B Intraspecific Sequence Divergence. Within the 297 bp segment of the cytochrome b region, 15 positions were variable among all samples (Appendix). With one exception, variable positions were transitions. There were 11 substitutions in third codon positions between North and South American Burrowing Owls. There was one substitution in the first position of a codon between North American Burrowing Owls (*A. c. hypugaea* and *A. c. floridana*) and *A. c. cunicularia* from Argentina, and two substitutions were in the first position of a codon between North American Burrowing Owls and *A. c. nanodes* and *A. c. punensis*, which resulted in one amino acid change (Appendix). The western (*A. c. hypugaea*) and Florida (*A. c. floridana*) Burrowing Owls each differed from the Peru (*A. c. nanodes*) and Ecuador (*A. c*

Table 2. Percent nucleotide distribution at first, second, and third codon positions of 297 bp cytochrome b fragment for Burrowing, Elf, Barred, and Eastern Screech Owls.

	FIRST				SECOND				THIRD			
	A	G	C	T	A	G	C	T	A	G	C	T
Athene cunicularia	23	26	24	27	16	20	27	34	26	7	59	7
Micrathene whitneyi	26	24	26	25	20	19	25	37	38	3	48	10
Strix varia	24	25	24	26	24	18	28	36	28	7	55	9
Otus asio	23	26	28	23	21	19	24	36	28	7	53	9

Table 3. Pairwise distances (based on cytochrome b data) among taxa. Above the diagonal are absolute distances and below the diagonal are total number of observed differences. Total number of observed differences equals number of transitions plus transversions; number of transversions are in parentheses.

TAXA	1	2	3	4	5	6	7	8
1. *A. c. hypugaea*	—	0.007	0.040	0.044	0.044	0138	0.185	0.178
2. *A. c. floridana*	2 (0)	—	0.040	0.044	0.044	0.138	0.178	0.178
3. *A. c. cunicularia*	12 (0)	12 (0)	—	0.010	0.010	0.135	0.168	0.168
4. *A. c. nanodes*	13 (1)	13 (1)	3 (0)	—	0.000	0.135	0.168	0.168
5. *A. c. punensis*	13 (1)	13 (1)	3 (0)	0 (0)	—	0.135	0.168	0.168
6. *Micrathene whitneyi*	41 (19)	41 (19)	40 (19)	40 (19)	40 (19)	—	0.135	0.152
7. *Otus asio*	55 (22)	55 (22)	50 (23)	50 (23)	50 (23)	40 (16)	—	0.135
8. *Strix varia*	53 (19)	53 (19)	50 (16)	50 (16)	51 (16)	45 (19)	40 (18)	—

punensis) forms by 12 transitions and one transversion (percent divergence: $P = 4.4$) and the Argentinean form (*A. c. cunicularia*) by 12 transitions ($P = 4.0$). The western and Florida Burrowing Owls differed by two transitions (percent nucleotide divergence: $P = 0.7$). The Argentinean Burrowing Owls (*A. c. cunicularia*) differed from Burrowing Owls of coastal Ecuador (*A. c. punensis*) and Peru (*A. c. nanodes*) by three transitions ($P = 1.0$), and no differences were observed between *A. c. nanodes* and *A. c. punensis* (Table 3).

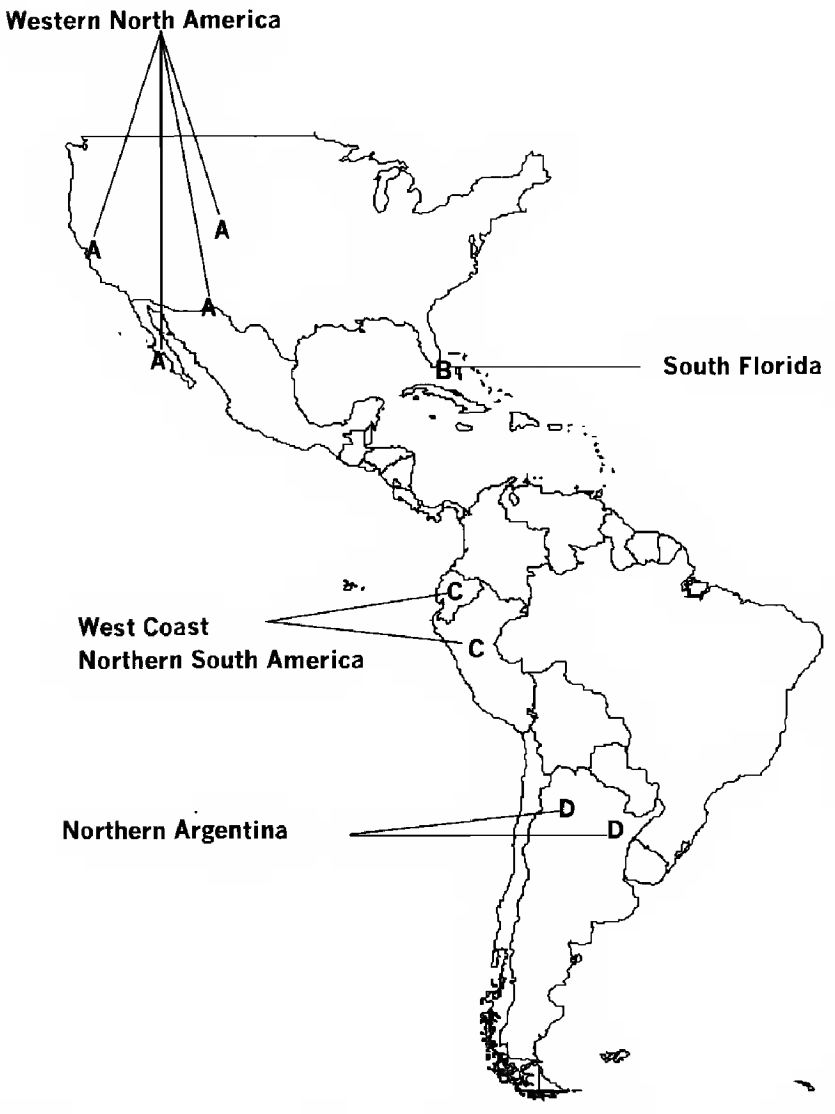

Figure 1. Geographic localities of the distinct mtDNA genotypes: A = *Athene cunicularia hypugaea,* B = *A. c. floridana,* C = *A. c. nanodes* and *A. c. punensis,* D = *A. c. cunicularia.*

Rates of Evolution. Wood and Krajewski (1996) estimated the rate of evolution of cytochrome b sequences for cranes (*Sarus* spp.) to be 1.7% per 1 million yr, lending support to the molecular clock calibration of 2% sequence divergence per million yr by Brown et al. (1982). Applying this conventional mammalian mtDNA molecular clock calibration of 2% sequence divergence per million yr to the cytochrome b sequence data, we estimate that the North and South American forms of the Burrowing Owl diverged ca. 2 million yr ago. Of the three South American subspecies examined, *A c. cunicularia* diverged from *A. c. punensis* and *A. c nanodes* ca. 500 000 yr ago, and coastal populations representing *A. c. nanodes* and *A. c. punensis* of Peru and Ecuador showed no evidence of divergence. The two North American subspecies (*A. c. hypugaea* and *A. c. floridana*) diverged more recently, ca. 350 000 yr ago (Fig. 1). This molecular clock has been widely applied to various taxa; however, given uncertainties about mtDNA clocks in birds and other taxa (Rising and Avise 1993), this estimate should be cautiously interpreted. Rates of evolution were not determined among owl species using cytochrome b data due to the saturation of nucleotide substitutions.

DISCUSSION

Cytochrome b Sequences. The cytochrome b sequences obtained for Burrowing Owls and other owl species were in agreement with other published avian cytochrome b sequences (Kocher et al.

1989, Birt-Friesen et al. 1992, Wood and Krajewski 1996). Like other avian cytochrome b sequences, all four owl species exhibited a deficiency of guanine. When nucleotide distribution was examined in relation to codon position, the four owl sequences exhibited a pattern similar to other avian taxa, exhibiting a deficiency for both guanine and thymine at third codon positions. The guanine deficiency also has been reported in rodents and fish (Kocher et al. 1989). A deficiency in both guanine and thymine at the third codon position has only been reported for avian species (Kocher et al. 1989, Quinn and Wilson 1993).

The level of variability detected within and between Burrowing Owl populations from North and South America, using cytochrome b, is higher than, but consistent with, the level of cytochrome b variability detected in other avian studies that make intraspecific comparisons (Edwards and Wilson 1990, Wenink et al. 1993). The agreement in observed nucleotide composition with other avian species, and the similarity in levels of observed variability within other avian species suggests that the cytochrome b sequence data for the four owl species is authentic mitochondrial cytochrome b sequence. In addition, the amino acid translations for the nucleotides did not reveal any stop codons interrupting translation that would indicate a nonfunctional copy.

Distribution and Divergence of Cytochrome b Sequences. Cytochrome b sequence results revealed a clear split between Burrowing Owls from North and South America (4.0–4.4% divergence). The sequence data contained numerous diagnostic sites for North and South American Burrowing Owls. The depth of this split is consistent with species-level distinction that has been described in other studies using this molecular marker (Edwards and Wilson 1990, Birt-Friesen et al. 1992, Wood and Krajewski 1996) and studies using restriction endonucleases on mtDNA (Kessler and Avise 1984, Mack et al. 1986, Shields and Wilson 1987). Within-genus sequence divergence among *Sarus* cranes (Wood and Kajewski 1996) is similar to divergence values between North and South American populations of Burrowing Owls. Several studies examining sibling taxa (Kessler and Avise 1984, Shields and Wilson 1987, Avise and Zink 1988) report lower values for genetic distances than observed between North and South American forms of the Burrowing Owl. The one notable exception is the study on Australian Babblers (*Pomatostomus temporalis* Edwards and Wilson 1990), which reports large within-subspecies values for genetic distance using cytochrome b. They report a mean of 1.4% and 1.3% within northern and southern groups of *P. temporalis*, respectively, and a mean of 3.2% between the northern and southern forms. They comment that this degree of divergence is large for subspecific status and suggest further investigation into the specific status of the bird. The even greater mtDNA distances between the North and South American forms of the Burrowing Owl reported here suggest that the species status of these owls should also be reevaluated. The distances observed between *A. c. hypugaea* and *A. c. floridana* for North America and between *A. c. cunicularia* and *A. c. nanodes/punensis* for South America are consistent with subspecies-level distinctions that have been observed in other studies.

Although a strong split between Burrowing Owls from North and South America was detected, we cannot geographically define exactly where the split occurs because of our limited sampling. It most likely occurs in Central America or extreme northern South America (i.e., Colombia). A more intensive sampling effort between the areas in question would determine whether or not this break is between two distinct clades.

Biogeographic Patterns. The divergence of North and South American forms of the Burrowing Owl ca. 2 million yr ago coincides with the presence of the isthmian land bridge, which arose at that time, providing a dispersal corridor between continents. The mingling of the North and South American faunas is postulated to have spanned a 2 million yr period between the last million years of the Pliocene and the first million years of the Pleistocene (from 3 until 1 million yr ago). Webb (1978) estimated that a large portion (2/3 for mammals) of the faunal exchange involved savanna-adapted organisms. The presence of the Burrowing Owl ancestor in the North American fossil record dating back to the Pliocene, and South American fossil records of the Burrowing Owl from the Pleistocene, may indicate that these birds evolved in North America and subsequently dispersed to the South American continent; however, the sparse fossil record may be misleading. The climatic fluctuations in South America associated with glacial and interglacial periods resulted in isolated patches of suitable habitat that ranged from small to large expanses of grasslands and savanna-type habitats. The disjunct distribution of

suitable habitat throughout South America helps explain the diversity among Burrowing Owl populations on that continent (11 recognized subspecies). The genetic divergence observed between cytochrome b sequences of Burrowing Owls from North and South America is large and warrants further investigation regarding full species status. Verification of the observed cytochrome b sequence divergence with nuclear markers, morphology and/or other ecological indicators, such as vocalizations or behavior, is needed.

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LITERATURE CITED

AVISE, J.C. AND R.M. ZINK. 1988. Molecular genetic divergence between avian sibling species: King and Clapper Rails, Long-billed and Short-billed Dowitchers, Boat-tailed and Great-tailed Grackles, and Tufted and Black-crested Titmice. *Auk* 105:516–528.

BIRT-FRIESEN, V.L., W.A. MONTEVECCHI, A.J. GASTON, AND W.S. DAVIDSON. 1992. Genetic structure of thick-billed murre (*Uria lomvia*) populations examined using direct sequence analysis of amplified DNA. *Evolution* 46: 267–272.

BROWN, W.M., E.M. PRAGER, A. WANG, AND A.C. WILSON. 1982. Mitochondrial DNA sequences of primates: tempo and mode of evolution. *J. Mol. Evol.* 18:225–239.

DESMOND, M.J. 1997. Evolutionary history of the genus *Speotyto*: a genetic and morphological perspective. Ph.D. dissertation, Univ. Nebraska, Lincoln, NE U.S.A.

EDWARDS, S.V. AND A.C. WILSON. 1990. Phylogenetically informative length polymorphism and sequence variability in mitochondrial DNA of Australian songbirds (*Pomatostomus*). *Genetics* 126:695–711.

FORD, N.L. 1966. Fossil owls from the Rexroad Fauna of the upper Pliocene of Kansas. *Condor* 68:472–475.

HAFFER, J. 1974. Avian speciation in tropical South America. *In* R.A. Paynter, Jr. [ED.], Publ. Nuttall Ornithol Club No. 14. Cambridge, MA U.S.A.

JOHNSGARD, P.A. 1988. North American owls. Smithsonian Instit. Press, Washington, DC U.S.A.

KESSLER, L.G. AND J.C. AVISE. 1984. Systematic relationships among waterfowl (Anatidae) inferred from restriction endonuclease analysis of mitochondrial DNA. *Syst. Zool.* 33:370–380.

KOCHER, T.D., W.K. THOMAS, A. MEYER, S.V. EDWARDS, S. PAABO, F.X. VILLABLANCA, AND A.C. WILSON. 1989. Dynamics of mitochondrial DNA evolution in animals amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci. U.S.A.* 86:6196–6200.

MACK, A.L., F.B. GILL, R. COLBURN, AND C. SPOLSKY. 1986 Mitochondrial DNA: a source of genetic markers for studies of similar passerine bird species. *Auk* 103:676–681.

PETERS, J.L. 1940. Check-list of birds of the world. Vol. 4 Harvard Univ. Press, Cambridge, MA U.S.A.

PREGILL, G.K. AND S.L. OLSON. 1981. Zoogeography of West Indian vertebrates in relation to Pleistocene climatic changes. *Annu. Rev. Ecol. Syst.* 12:75–98.

QUINN, T.W. AND A.C. WILSON. 1993. Sequence evolution in and around the mitochondrial control region in birds. *J. Mol. Evol.* 37:417–425.

RISING, J.D. AND J.C. AVISE. 1993. Application of genealogical-concordance principles to the taxonomy and evolutionary history of the Sharp-tailed Sparrow (*Ammodramus caudacutus*). *Auk* 110:844–856.

SHIELDS, G.F. AND A.C. WILSON. 1987. Subspecies of the Canada Goose (*Branta canadensis*) have distinct mitochondrial DNA's. *Evolution* 4:662–666.

SWOFFORD, D.L. AND D.P. BEGLE. 1993. PAUP: phylogenetic analysis using parsimony, version 3.1. Illinois Natural History Survey, Champaign, IL U.S.A.

VUILLEUMIER, F. 1985. Fossil and recent avifaunas and the interamerican interchange. *In* F.G. Stehli and S.D Webb [EDS.], The Great American Biotic Interchange. Plenum Press, New York, NY U.S.A.

WEBB, S.D. 1977. A history of savanna vertebrates in the New World. Part I: North America. *Annu. Rev. Ecol Syst.* 8:355–380.

———. 1978. A history of savanna vertebrates in the New World. Part II: South America and the Great Interchange. *Annu. Rev. Ecol. Syst.* 9:393–426.

WENINK, P.W., A.J. BAKER, AND M.G.J. TILANUS. 1993. Hypervariable-control-region sequences reveal global population structuring in a long-distance migrant shorebird, the Dunlin (*Calidris alpina*). *Proc. Natl. Acad. Sci. U.S.A.* 90:94–98.

WOOD, T.C. AND C. KRAJEWSKI. 1996. Mitochondrial DNA sequence variation among the subspecies of Sarus Crane (*Grus antigone*). *Auk* 113:655–663.

Appendix. Nucleotide and inferred amino acid sequences of 297 bp fragment of the cytochrome b gene for Burrowing Owl subspecies and the Elf, Eastern Screech, and Barred Owls. Sequence orientation is from 5′ to 3′ on the light strand. Dots indicate identity with *A. c. hypugaea* sequences, and dashes indicate gaps in sequences. Sites in bold are amino acid replacements within Burrowing Owls, and sites in italics indicate amino acid replacements among owl species.

A. c. hypugaea[a]	C	TTC	GGA	TCC	CTG	CTA	GGC	ATC	TGC	TTG	ACA	ACT	CAG	ATC	ATT
A. c. hypugaea[b]	.	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A c. hypugaea[c]	.	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A c. floridana[d]	.	...	...	...	...	...	...	...	...	...	...	..C	...	...	...
A c. cunicularia[e]	.	...	...	...	..A	...	...	...	...	...	...	..C	...	...	...
A c. cunicularia[f]	.	...	...	...	..A	...	...	...	...	...	...	..C	...	...	...
A c. nanodes[g]	.	...	...	...	..A	...	...	...	...	...	...	..C	...	...	...
A c. punensis[h]	.	...	...	...	..A	...	...	...	...	...	...	..C	...	...	...
Micrathene whitneyi	.	...	...	..T	..A	..T	...	...	...	C.A	.T.	..C	..A	...	..C
Otus asio	.	...	..G	...	...	..T	..A	..T	...	C.A	.T.	G.C	..A	...	..C
Strix varia	.	...	..G	...	..A	T..	..A	...	..T	C.A	G..	..C	..A	...	..C
		phe	gly	ser	leu	leu	gly	ile	cys	leu	*thr*	*thr*	gln	ile	ile

A c. hypugaea	ACT	GGC	CTC	TTA	CTA	GCC	ACC	CAC	TAC	ACA	GCC	GAC	TCC	TCC
A. c. hypugaea	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A. c. hypugaea	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A. c. floridana	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A c. cunicularia	...	...	...	C..	...	...	...	...	...	...	...	...	...	...
A c. cunicularia	...	...	...	C..	...	...	...	...	...	...	...	...	...	...
A c. nanodes	...	...	...	C..	...	...	...	...	...	...	...	...	...	...
A. c. punensis	...	...	...	C..	...	...	...	...	...	...	...	...	...	...
Micrathene whitneyi	...	...	...	C..	...	...	..A	..T	...	...	A.T	...	..A	A..
Otus asio	..A	...	...	C..	..C	...	..A	...	..T	...	...	...	..A	A..
Strix varia	...	...	...	C.C	..T	..A	G..	...	..T	...	...	...	..A	G..
	thr	gly	leu	leu	leu	ala	*thr*	his	tyr	thr	*ala*	asp	ser	*ser*

A. c. hypugaea	CTG	GCC	TTC	ACA	GCT	GTC	TCA	CAC	ACA	TGC	CGA	GAC	GTC	CAA
A c. hypugaea	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A c. hypugaea	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A c. floridana	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A c. cunicularia	..A	...	...	...	..C	...	...	...	...	...	...	...	...	...
A c. cunicularia	..A	...	...	...	..C	...	...	...	...	...	...	...	...	...
A c. nanodes	..A	...	...	...	...	...	...	...	...	...	...	A..	...	...
A c. punensis	..A	...	...	...	...	...	...	...	...	...	...	A..	...	...
Micrathene whitneyi	..A	...	..T	...	T.A	..A	..C	..T	...	...	...	...	...	...
Otus asio	..A	..A	...	...	T.C	..A	...	...	..C	...	...	..T	..G	...
Strix varia	..A	...	...	G..	..C	..A	..C	...	..C	...	...	A..	A..	...
	leu	ala	phe	*thr*	*ala*	val	ser	his	thr	cys	arg	***asp***	*val*	gln

A c. hypugaea	TAC	GGC	TGA	CTC	ATC	CGC	AAC	CTC	CAT	GCA	AAC	GGG	GCA	TCC
A c. hypugaea	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A c. hypugaea	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A c. floridana	..T	...	...	...	...	...	...	...	...	...	...	...	...	...
A c. cunicularia	...	...	...	...	...	...	...	...	..C	...	...	..A	...	...
A c. cunicularia	...	...	...	...	...	...	...	...	..C	...	...	..A	...	...
A c. nanodes	...	...	...	...	...	...	...	...	..C	...	...	..A	...	...
A. c. punensis	...	...	...	...	...	...	...	...	..C	...	...	..A	...	...
Micrathene whitneyi	...	..A	...	..A	...	...	...	...	...	...	..T	..A	..C	..A
Otus asio	..T	...	...	..A	C.A	...	...	...	..C	..G	..T	..A	..C	..A
Strix varia	...	...	...	...	..A	...	...	...	..C	...	...	...	..C	..A
	tyr	gly	trp	leu	*ile*	arg	asn	leu	his	ala	asn	gly	ala	ser

Appendix. Continued.

A. c. hypugaea	ATA	TTC	TTT	ATC	TGC	ATC	TAC	CTC	CAC	ATC	GGA	CGA	GGC	CTA
A. c. hypugaea	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A. c. hypugaea	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A. c. floridana	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A. c. cunicularia	..G	...	..C	...	...	...	...	...	...	..T	...	..G	...	T..
A. c. cunicularia	..G	...	..C	...	...	...	...	...	...	..T	...	..G	...	T..
A. c. nanodes	..G	...	..C	...	...	...	...	...	...	..T	...	..G	...	T..
A. c. punensis	..G	...	..C	...	...	...	...	...	...	..T	...	..G	...	T..
Micrathene whitneyi	..C	...	...	...	...	...	...	..A	...	...	...	...	...	A..
Otus asio	..T	...	..C	...	...	...	...	..A	...	...	...	..G	...	...
Strix varia	C.C	...	..C	...	...	...	...	..G	...	...	...	...	...	...
	ile	phe	phe	ile	cys	ile	tyr	leu	his	ile	gly	arg	gly	*leu*
A. c. hypugaea	TAC	TAC	GGC	TCA	TAC	CTC	TAC	AAA	GAA	ACC	TGA	AAC	ACA	GGT
A. c. hypugaea	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A. c. hypugaea	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A. c. floridana	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A. c. cunicularia	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A. c. cunicularia	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A. c. nanodes	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A. c. punensis	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Micrathene whitneyi	...	...	...	..T	...	...	...	...	...	...	...	...	...	..A
Otus asio	...	...	...	..C	...	..A	...	...	...	...	...	...	...	...
Strix varia	...	...	...	..C	..T	..G	...	...	..G	...	...	..T	...	...
	tyr	tyr	gly	ser	tyr	leu	tyr	lys	glu	thr	trp	asn	thr	gly
A. c. hypugaea	GTC	CTA	CTT	CTC	TTG	ACC	CTA	ATA	GCC	ACC	GCC	TTC	GTG	GGC
A. c. hypugaea	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A. c. hypugaea	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A. c. floridana	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A. c. cunicularia	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A. c. cunicularia	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A. c. punensis	...	...	...	...	...	...	..G	...	...	...	...	...	...	...
A. c. nanodes	...	...	...	...	...	...	..G	...	...	...	...	...	...	...
Micrathene whitneyi	...	N..	..C	...	C..	...	...	...	..A	..A	..N	...	...	...
Otus asio	...	...	..C	..A	C..	...	..T	...	..A	..T	..T	...	...	...
Strix varia	A.T	...	T.A	...	...	...	...	...	..T	...	...	..A	...	...
	val	leu	leu	leu	leu	thr	leu	ile	ala	thr	ala	*phe*	val	gly
A. c. hypugaea	TA													
A. c. hypugaea	..													
A. c. hypugaea	..													
A. c. floridana	..													
A. c. cunicularia	..													
A. c. cunicularia	..													
A. c. nanodes	..													
A. c. punensis	..													
Micrathene whitneyi	..													
Otus asio	..													
Strix varia	..													

[a] Collected in Baja California, Mexico.
[b] Collected in western Nebraska, U.S.A.
[c] Collected in central California, U.S.A.
[d] Collected in southern Florida, U.S.A.
[e] Collected in Providence Corrientes, Argentina.
[f] Collected in Providence Tucuman, Argentina.
[g] Collected in Providence Trujulio, Peru.
[h] Collected in Providence Loja, Ecuador.